Pedobiologia **43**, 625–629 (1999)
Urban & Fischer Verlag
http://www.urbanfischer.de/journals/pedo

Pedo biologia

Enchytraeid Reproduction Test (ERT): Sublethal responses of two *Enchytraeus* species (Oligochaeta) to toxic chemicals

Rut Collado[1], Rüdiger M. Schmelz[1,3], Thomas Moser[2] and Jörg Römbke[2]

[1] University of Osnabrück, FB5, Systematic Zoology, D-49069 Osnabrück, Germany
[2] ECT Oekotoxikologie GmbH, Böttgerstr. 2–14, D-65439 Flörsheim, Germany

Accepted: 25. March 1999

Summary. Recently, the ERT (Enchytraeid Reproduction Test) was developed as a new laboratory test system using species of the genus *Enchytraeus* (mainly *E. albidus*). The main endpoint is reproduction (= number of juveniles), not mortality. Since *E. albidus*, which was chosen mainly for its size, prefers organic substrates, the test was also performed with another, true soil-dwelling species of the same genus. The species belongs to the *E. buchholzi/minutus* – complex, and is smaller and reproduces faster than *E. albidus*. Each species was tested for its sublethal susceptibility to carbendazim (formulation Derosal®) and 4-nitrophenol, respectively. The tests were performed following the Draft Guideline of the Enchytraeid Reproduction Test (ERT).

The NOEC value (reproduction) for carbendazim was determined as 1.2 mg/kg (*E. albidus*) and 2.7 mg/kg (*E. "buchholzi"*), whereas the NOEC values calculated for 4-nitrophenol were10.0 mg/kg (*E. albidus*) and 5.6 mg/kg (*E. "buchholzi"*). Neither of the examined species proved to be more susceptible to these chemicals than the other in general.

Key words: Enchytraeidae, *Enchytraeus* sp., ecotoxicology, laboratory test, carbendazim, 4-nitrophen

Introduction

Recently, the ERT (Enchytraeid Reproduction Test) was developed as a new laboratory test system using species of the genus *Enchytraeus* (mainly *E. albidus*). As in the well-known earthworm test with *Eisenia fetida* (OECD 1984, BBA 1994, ISO 1995), artificial soil is used as the test substrate, but the main endpoint is reproduction (= number of juveniles), not mortality. Since *E. albidus*, which was chosen mainly for its size, is most often found at sites with a high organic content (compost heaps and marine shore debris) and only rarely in soils (Kasprzak 1982), the test was also performed with another, true soil-dwelling species of the same genus.

In this contribution we present the results of an ecotoxicological comparison between *E. albidus* and an up-to-now undescribed soil-dwelling species from the *E. buchholzi/minutus*-species complex (Schmelz et al. 1999), called *E. "buchholzi"* in the following. The soil-dwelling *Enchytraeus*-species is smaller and reproduces faster than *E. albidus*. It is widespread in meadow soils (at least) in Northern Germany (Schmelz pers. obs.). Each species was tested

---

[3] *Corresponding author. e-mail:* schmelz@biologie.uni-osnabrueck.de

0031-4056/99/43/06-625 $ 12.00/0

for its sublethal susceptibility to the fungicide carbendazim (formulation Derosal®) and the environmental chemical 4-nitrophenol, respectively. The aim of this study was to compare the reactions of these closely related species to these two chemicals, using the same test system.

Materials and Methods

The tests were performed following the Draft Guideline of the Enchytraeid Reproduction Test (ERT), which is based on a combined mortality/reproduction test developed nearly ten years ago (Römbke 1991). This draft guideline was recently validated in an international ringtest (Römbke & Moser 1999). Details of the test performance are given in Tab. 1. In total, four test runs were performed. The effects of carbendazim and 4-nitrophenol on the mortality and the reproduction of both test species were investigated. Normality and variance homogeneity of the test results were assessed using Kolmogoroff-Smirnov's test and Bartlett's test, respectively. Multiple t-tests such as the Williams test were performed with normally distributed and homogeneous data.

Table 1. Short characterisation of the ERT (Enchytraeid Reproduction Test)

Subject	*Description*
Guideline:	Draft guideline according to OECD standards
Test species:	*Enchytraeus albidus*; *Enchytraeus* sp.; 10 adult worms (as identified by visible eggs in the clitellum region)
Substrate:	Artificial soil according to OECD (1984)
Test Design and Duration:	Range-finding test: 2 weeks. Definitive 2est: *E. albidus*: 6 weeks. Removal of the adult worms after 3 weeks; counting of the juveniles hatched after 3 more weeks. *E. "buchholzi"*: 3 weeks. No removal of adults
Parameter:	Range-finding-test: mortality, behaviour Definitive test: number of juveniles
Performance:	Room temperature (20 ± 2 °C); weekly feeding (oats strewn onto the soil surface);
Concentrations:	Range-finding-test: Control, 0, 0.1, 1, 10, 100, 1000 mg/kg (1 replicate); Definitive Test: 5 concentrations plus control (4 replicates)
Test substances:	Fungicide carbendazim (formulation Derosal®) Industrial chemical 4-nitrophenol
Data assessment:	Determination of the No-Observed-Effect-Concentration (NOEC) by multiple t-tests
Validity criteria:	Adult mortality in the control less than 20% Number of juveniles in the control more than 25 per 10 adults Coefficient of variation (reproduction) less than 50%

From various studies the fungicide carbendazim is known as very toxic for oligochaetes (Stringer & Wright 1976; Lofs-Holmin 1981; Römbke 1989; Federschmidt 1994). Carbendazim is the first metabolite of the widely used fungicide benomyl, which is also very toxic for oligochaetes. The environmental chemical 4-nitrophenol is the metabolite of the widely used insecticide parathion. Both are only moderately toxic for oligochaetes (Neuhauser et al. 1986; Römbke et al. 1994).

Results and Discussion

In nearly all test runs the mortality did not exceed 20%. Only in the carbendazim test with *E. albidus* was the mortality criterion very slightly missed (Tab. 2). The rate of mortality was lower in both tests with *E. "buchholzi"*. All test runs can be regarded as valid, as the mean number of juveniles in the controls was always higher than 25 (61 and 69 for *E. albidus*, 214 and 165 for *E. "buchholzi"*) and the coefficient of variation was always lower than 50% (Tab. 2).

Table 2. Values of the validity criteria in the four tests performed with *E. albidus* and *E. "buchholzi"*, respectively

	Validity criterion	*E. albidus*	*E. "buchholzi"*
Carbendazim tests	Adult mortality (%)	20.0	12.5
	Number of juveniles	60.6	213.6
	Coeff. of variation (%)	23.6	16.9
4-nitrophenol tests	Adult mortality (%)	21.2	5.0
	Number of juveniles	69.3	161.5
	Coeff. of variation (%)	29.3	9.7

The number of juveniles is shown in Fig. 1 (carbendazim) and Fig. 2 (4-nitrophenol). The NOEC values (reproduction) for carbendazim were determined as 1.2 mg/kg (*E. albidus*) and 2.7 mg/kg (*E. "buchholzi"*) (Tab. 3). Although *E. albidus* is larger than *E. "buchholzi"* it reacts slightly more sensitively to carbendazim. The NOEC values calculated for 4-nitrophenol were 10.0 mg/kg (*E. albidus*) and 5.6 mg/kg (*E. "buchholzi"*). Here the larger species appears to be less sensitive than the smaller one.

With respect to the high variance of the individual test results, no statistical comparison of the NOEC values was performed. In general, neither of the examined species proved to be

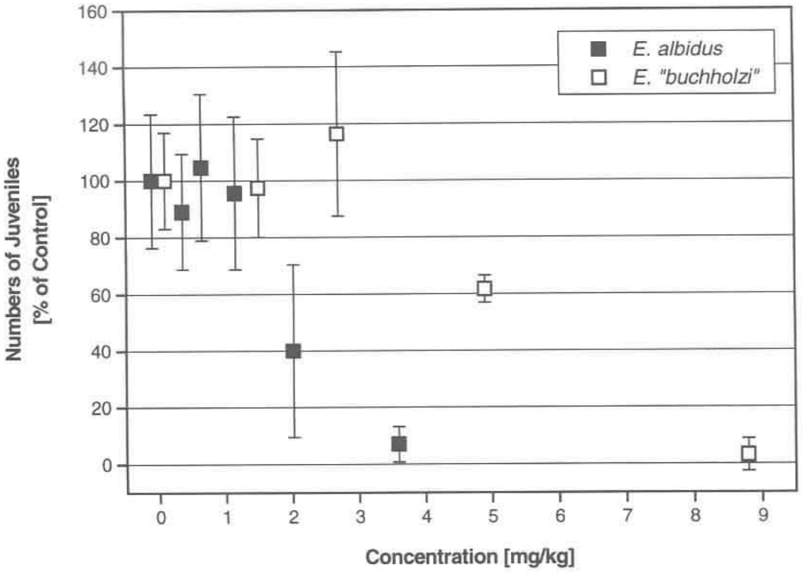

Fig. 1. Effects of carbendazim (active ingredient) on the number of juveniles in two test runs performed with *E. albidus* and *E. "buchholzi"*, respectively (4 replicates per species and concentration, bars = SD)

Table 3. NOEC vor reproduction (mg/kg) determined for carbendazim and 4-nitrophenol in the tests with *E. albidus* and *E. "buchholzi"*

	E. albidus	*E. "buchholzi"*
Carbendazim	1.2	2.7
4-nitrophenol	10.0	5.6

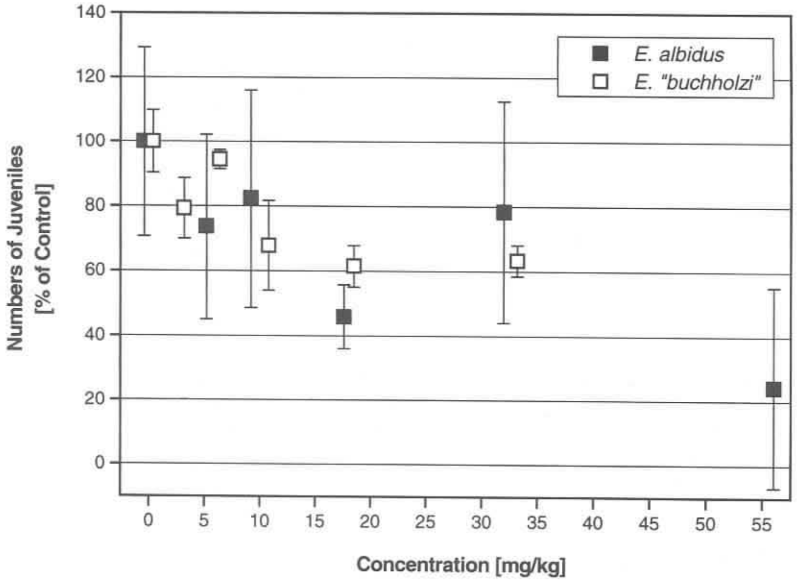

Fig. 2. Effects of 4-nitrophenol (active ingredient) on the number of juveniles in two test runs performed with E. albidus and *E. "buchholzi"*, respectively (4 replicates per species and concentration, bars = SD)

more susceptible to chemicals than the other because inter-specific sensitivity differences like those found here can be considered to be within the same range (Smrchek & Zeeman 1998). Our findings as well as published data (e.g. Neuhauser et al. 1986; Federschmidt 1994) confirm the statement of Cairns (1986) that there is not one "most sensitive species".

The observed differences are also relatively small when taking the further use of such ecotoxicological test results into consideration. Usually these data are required as part of the risk assessment process when registering pesticides or notifying environmental chemicals. In such a process NOEC values are divided by high unsafety factors (e.g. 10) to take into account that single-species-tests do not cover all species, all possible endpoints and cannot represent the ecosystem to be protected. The NOEC value divided by an unsafety factor is called PNEC (Predicted-No-Effect-Concentration). PNEC values are compared with PEC values (Predicted-Exposure-Concentration) for the decision whether a chemical might have an ecotoxicological hazard potential or not.

Coming back to our results, it appears that the value of the unsafety factor used in the risk assessment process (10) is higher than the differences in sensitivity observed in our real tests (ca. 2). However, the experiences gained with two species from the same genus and only two chemicals do not allow us yet to formulate a generally valid conclusion regarding sensitivity differences. Further tests following the same standardised protocol will be necessary to show how large sensitivity differences are on average and whether these differences have to be accounted for in the risk assessment process in another way than it is done today (e.g. by changing the extrapolation factors used so far).

Acknowledgements

We thank the German Federal Environmental Agency (Berlin, Germany) and the European Chemical Bureau (Ispra, Italy) for funding the studies reported here. Especially we would like to thank Prof. Dr. H. T. Ratte (University of Aachen, Germany) for statistical advice.

References

BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) (1994) Richtlinienvorschlag für die Prüfung von Pflanzenschutzmitteln (Nr. VI, 2–2): Auswirkungen von Pflanzenschutzmitteln auf die Reproduktion und das Wachstum von *Eisenia fetida/Eisenia andrei*.

Cairns, J. (1986) The myth of the most sensitive species. Bioscience 36, 670–672.

Federschmidt, A. (1994) Die Oligochaetenfauna zweier Ökosysteme auf Lößlehm unter Berücksichtigung der Auswirkungen von Chemikalienstreß. Dissertation Universität Frankfurt.

ISO (International Organisation for Standardisation) (1995) Soil Quality – Effects of Pollutants on Earthworms (*Eisenia fetida*). Part 2: Determination of Effects on Reproduction. Draft International Standard ISO DIS 11268-2.

Kasprzak, K. (1982) Review of enchytraeid community structure and function in agricultural ecosystems. Pedobiologia 23, 217–232.

Lofs-Holmin, A. (1981) Influence in field experiments of Benomyl and carbendazim on earthworms (Lumbricidae) in relation to soil texture. Swedish Journal of Agricultural Research 11, 141–147.

Neuhauser, E. F., Durkin, P. R., Malecki, M. R., Anatra, M. (1986) Comparative toxicity of ten organic chemicals to four earthworm species. Comparative Biochemistry and Physiology 83C, 197–200.

OECD (Organisation for Economic Development) (1984) OECD-Guideline for Testing of Chemicals No. 207. Earthworm Acute Toxicity Test. Paris.

Römbke, J. (1989) *Enchytraeus albidus* (Enchytraeidae, Oligochaeta) as a test organism in terrestrial laboratory systems. Archives of Toxicology 13, 402–405.

Römbke, J. (1991) Entwicklung eines Reproduktionstests an Bodenorganismen – Enchytraeen. Bericht im Auftrag des Umweltbundesamts, F+E-Vorhaben Nr. 106 03 051/01, Battelle Institut Frankfurt.

Römbke, J., Knacker, T., Förster, B., Marcinkowski, A. (1994) Comparison of effects of two pesticides on soil organisms in laboratory tests, microcosms, and in the field. In: Donker, M.H., Eijsakers, H., Heimbach, F (eds.) Ecotoxicology of Soil Organisms. Lewis Publishers, Chelsea, Michigan, 229–240.

Römbke, J., Moser, T. (1999) Organisation and performance of an international ringtest for the validation of the Enchytraeid Reproduction Test. Vol. I, II. (Umweltbundesamt Texte, 4.) Federal Environmental Agency (Umweltbundesamt) Research Report 29664906, UBA-FB 98-104, Berlin. 150 + 224 pp.

Schmelz, R. M., Collado, R., Oestreich, M. (1999) Taxonomy of the *Enchytraeus minutus/buchholzi* species complex – outline of a problem and first steps towards a solution. Newsletter on Enchytraeidae 6, 31–39.

Smrchek, J. R., Zeeman, M. G. (1998) Assessing risks to ecological systems from chemicals. In: Calow, P. (ed) Handbook of Environmetal Risk Assessment and management. Blackwell Scientific Publications, Oxford, 24–90.

Stringer, A., Wright, M. A. (1976) The toxicity of benomyl and some related 2-substituted benzimidazoles to the earthworm *Lumbricus terrestris*. Pesticide Science 7, 459–464.